

Evidence for metapopulation structure in seep-dwelling amphipods

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Academic editor: Fabio Stoch | Received 23 March 2023 | Accepted 2 May 2023 | Published 4 May 2023

<https://zoobank.org/385F0F62-D59F-4E4F-88A8-1A53E8B7A8FA>

Citation: Culver DC, Feller DJ, Burch E (2023) Evidence for metapopulation structure in seep-dwelling amphipods. Subterranean Biology 45: 157–164. <https://doi.org/10.3897/subtbiol.45.103939>

Abstract

Twelve seepage springs in a 3 km² area of the Goldmine Tract in the C&O Canal National Historical Park in Montgomery County, Maryland, USA were sampled in 1994–1995 and again in 2023 for three amphipod species: *Stygobromus tenuis potomacus*, *Stygobromus pizzinii*, and *Crangonyx shoemakeri*. During that time interval, there were 11 colonizations and 1 extinction. Three populations persisted. These results are consistent with the proposed metapopulation hypothesis of population structure.

Keywords

Hypotelminorheic, springs, stygobionts, subterranean, urban conservation

Introduction

The most superficial shallow subterranean aquatic habitat, the hypotelminorheic, first described in the Medvednica Mountain of Croatia by Meštrov (1962), remains enigmatic. As described by Meštrov (1962, 1964), and later elaborated by Culver et al. (2006) and Pipan et al. (2012), it is a miniaturized subterranean drainage basin, underlain by clay, with a surface projection of about one hectare (see Staley 2016). The depth of the clay layer from the surface varies, but typically is a meter or less. The surface

manifestation of the hypotelminorheic is a seep, or seepage spring. Seepage springs are usually little more than wet spots in the woods. They typically have low flow that may not be discernible, occur in a slight depression, and have blackened leaves. During the summer months, the seeps are generally dry, with the only water being that in the colloidal clays. Although similar habitats have been reported in grasslands and even in tile drains of cultivated fields (Koenemann and Holsinger 2001; Culver et al. 2012), most hypotelminorheic habitats are in forested areas.

Hypotelminorheic habitats have proved very difficult to sample and to date, only by direct examination of the leaves and sediment of the seepage spring (Culver et al. 2006) or eDNA analysis of water samples has proven effective (Niemiller et al. 2017). Furthermore, seepage springs can only be sampled from late fall (leaf fall) to early spring (leaf out). During the rest of the year, presumably most of the superficial groundwater is taken up by evapotranspiration.

In the Potomac River basin of eastern USA, the region from Washington, DC to Cumberland, MD (approximately 160 km) has been intensively studied (e.g., Feller 1997; Hobson 1997; Culver and Šereg 2004) for the past several decades. From this work, a number of conclusions and working hypotheses have been formulated, listed in order of decreasing certainty. First, the habitat harbors a rich fauna specialized for subterranean life. There are amphipod, isopod, and snail species, with reduced or absent pigment and eyes. The highlight of this assemblage in the Potomac River basin around Washington, DC are the six species of blind, depigmented amphipods in the genus *Stygobromus* (Pipan et al. 2012). Except for *S. pizzinii*, which is found in a few caves in Pennsylvania, none of the species are found in either surface habitats or caves. Second, hypotelminorheic habitats tend to be clustered, presumably where a superficial clay layer is present. Where they are present, they form a mosaic of polygons defined by the movement of shallow subsurface water. Third, the seeps are more than exit points where hypotelminorheic animals get washed out. Currents are too low and animals are too large for the presence of animals at seeps to be accidental. It is possible that occasional currents are strong enough to dislodge amphipods from seeps, but it is certainly rare at best. Fong (unpublished but summarized in Culver and Pipan [2014]) shows that the presence of *Stygobromus tenuis potomacus* in a seep near Pimmit Run in the George Washington Memorial Parkway, Virginia, is highly temperature dependent, and they are nearly absent at higher temperatures occurring in the summer. This suggests that they are foraging for food in the relatively resource rich seepage spring. Fourth, habitat quality and the likelihood of finding species specialized for seeps is correlated with canopy height and presumably the quality of the leaf litter in the habitat (Burch et al. 2022). Fifth, the curious finding that no basic physico-chemical factor was a good predictor of the presence of animals, suggested that these were metapopulations, where individual populations blink on and off through time (Keany et al. 2018). However, individual species showed significantly different physico-chemical niches, particularly with respect to temperature and conductivity.

Metapopulation dynamics, first elaborated by Levins (1969) and much elaborated since (Hanski and Simberloff 2007), proposes that populations be governed by the balance between immigration and extinction, as well as the balance between birth and

death rates of local subpopulations. A metapopulation structure for hypotelminorheic species is highly conjectural because of the lack of direct evidence, especially evidence that sub-populations blink on and off.

The purpose of this note is to provide evidence for population extinction and colonization in a series of hypotelminorheic habitats in the C&O Canal National Historical Park in Montgomery County, Maryland, USA, based on samples of the same seeps in 1994–5 and 2023, a period of 28–29 years.

Methods

In 1996, Feller (1997) sampled 12 seeps in an area of 1500 by 2100 m in the Gold Mine Tract (Fig. 1) near the Mather Gorge section of the Potomac River near Washington, D.C. This is a mature hardwood forest dominated by oaks (*Quercus*), poplar (*Populus*), and beech (*Fagus*), with abundant foot trails, both official and “social.” The area is bisected by the Gold Mine Loop Trail. The water coming from the seeps coursed through a thick leaf and woody detritus in natural channels, terminating in the C&O Canal. The geology of the site is mapped as schist-Wissahickon Formation, Marburg Schist. Fine grained mica was commonly observed in seep runs. A typical seep (#11) is shown in Fig. 2.

In 2023, all 12 seeps were relocated and resampled for the following amphipod species in the family Crangonyctidae:

- *Stygobromus pizzinii* (Shoemaker, 1938)
- *Stygobromus tenuis potomacus* (Holsinger, 1967)
- *Crangonyx shoemakeri* (Hubricht & Mackin, 1940)

S. pizzinii and *S. tenuis potomacus* are without eyes or pigment, and except for a handful of caves in Pennsylvania where *S. pizzinii* has been found, they are limited to seeps. *C. shoemakeri* is common in seeps but also occurs in wetlands (Zhang and Holsinger 2003; Keany et al. 2018).

Collections in 1994–5 and 2023 were made in the same way—the hand examination of the litter and gravels at the exit of the hypotelminorheic—the seep. Each seep was sampled for a minimum of 30 person minutes. The 1994–1995 samples were taken in March and April, and the 2023 samples were taken in February, all times of relatively cold water and well before leaf out.

Results

The results of the census are listed in Table 1. During the 2023 census, an additional seep was found, near seep #9. This new seep had *S. tenuis potomacus*.

Between 1996 and 2023, *S. pizzinii* colonized two seeps, and persisted in one. *S. tenuis potomacus* colonized two seeps, went extinct (or at least were undetectable)

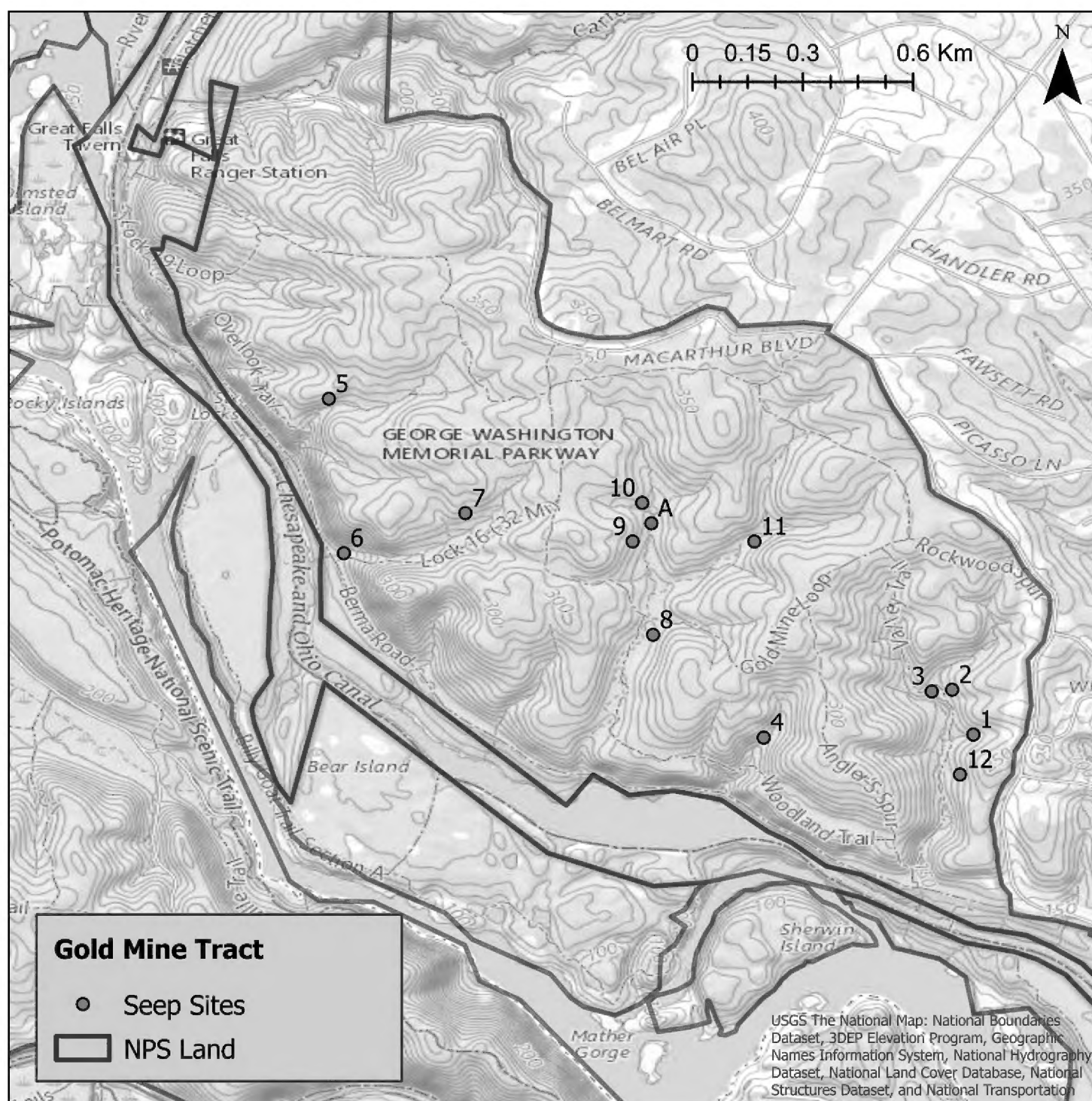


Figure 1. Map of study site. Numbers are from Feller (1997). The letter A indicates the location of a new seep discovered in 2023.

in one, and persisted in one. *C. shoemakeri* colonized six seeps and persisted in one. The one “new” seep (A in Fig. 1) had a population of *S. tenuis potomacus*. The major changes in 2023 was the occurrence of the six “new” seeps with *C. shoemakeri* and an increase in the number of seeps with detectable fauna by four.

Discussion

Our working hypothesis is that the seep dwelling amphipod populations form a metapopulation, as depicted schematically in Fig. 3. We have documented the changes that have occurred over a 25+ year period, and these include colonizations and extinctions, with more colonizations than extinctions (Table 1). While it is pos-



Figure 2. Seep 11 (see Fig. 1). Skunk cabbage (*Symplocarpus foetidus*) is growing around the seep, a frequent occurrence.

Table 1. Species composition of seeps in the study area (see Fig. 1) in 1994–5 and 2023. Numbers of individuals collected in 2023 are given in parentheses.

Seep No.	1994–5	2023
1	<i>S. pizzinii</i>	<i>S. pizzinii</i> (5), <i>S. tenuis potomacus</i> (3), <i>C. shoemakeri</i> (1)
2		
3		<i>S. tenuis potomacus</i> (2), <i>C. shoemakeri</i> (4)
4		<i>C. shoemakeri</i> (6)
5	<i>C. shoemakerii</i>	<i>S. pizzinii</i> (1), <i>C. shoemakeri</i> (4)
6		
7		
8	<i>S. tenuis potomacus</i>	<i>S. tenuis potomacus</i> (3), <i>C. shoemakeri</i> (6)
9		
10		<i>C. shoemakeri</i> (2)
11		<i>C. shoemakeri</i> (5)
12	<i>S. tenuis potomacus</i>	<i>S. pizzinii</i> (17)
A		<i>S. tenuis potomacus</i> (5)

sible that some populations were not detected during both censuses, it is also possible that more extinctions and recolonizations occurred when, for example, an extinction was followed by a recolonization during the rather long time period between sam-

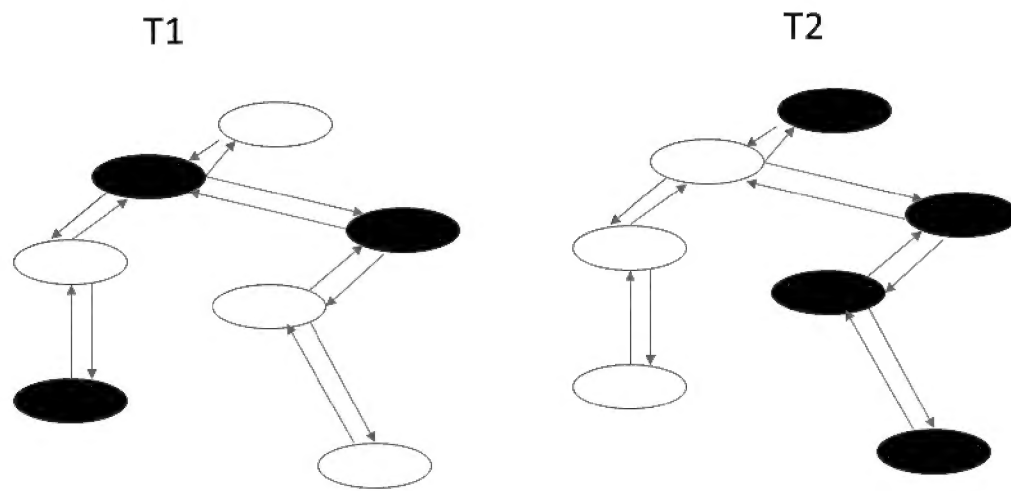


Figure 3. Representation of a metapopulation at two points in time. Black ellipses represent occupied seeps, white ellipses unoccupied seeps. Arrows are possible dispersal paths.

plings. Nonetheless, we believe the sampling resulted in the detection of many if not all populations.

The discovery of an additional seep in 2023 may be because it was not present in 1996. The hypotelminorheic habitat and the accompanying seep are transitory habitats, which exist almost exclusively in shallow soil and leaf litter. Minor alterations of the landscape, such as soil compaction, can change the location of seeps.

An especially pernicious problem in the study of the hypotelminorheic is the problem of false negatives. With the exception of eDNA, no technique has been found that is reliable as direct hand sampling (see Culver and Šereg 2004), and the success of hand sampling is somewhat dependent on flow rates of seeps. Flow rates in seeps have a complex relationship with precipitation since most precipitation is taken up by plant transpiration during the warmer months. Winter and spring precipitation (<https://www.weather.gov/media/lwx/climate/dcaprecip.pdf>), the time of maximum seep flow, was highest in 1994 (62.1 cm), lowest in 1995 (39.4 cm), and intermediate in 2022 and 2023 (49.8 cm), suggesting the changes we observed were not based on rainfall. A more accurate assessment would be to do repeated daily or weekly sampling, but this would be destructive to the habitat. Niemiller et al. (2017) demonstration of the feasibility of eDNA analysis to determine presence or absence of amphipods in seeps offers a way forward and an improvement on the present study. The only caveat is that the persistence time of eDNA in such subterranean habitats in general is unknown (Boulton et al. 2023). Finally, we note that our sample size was small ($n = 12$), not a statistically sufficient number for analysis. In spite of these caveats, we think the metapopulation hypothesis is worth pursuing.

A key remaining question is how migration and recolonization occur. Fong (quoted in Culver and Pipan [2014]) demonstrated that in a seep along Pimmit Run in the George Washington Memorial Parkway in Virginia, numbers of *S. tenuis potomacus* increased with decreasing temperature. This result implies that with cooler temperatures, the amphipods are more active foraging in the seep. Dispersal may occur when conditions are wet (and cool) enough. Water, usually storm runoff, in a thin layer over the ground surface, may be especially important. Sheet flow provides a temporary dispersal corridor both for active and passive dispersal.

While dispersal via groundwater is possible, groundwater is typically many meters below the hypotelminorheic. At least *S. tenuis potomacus* and *C. shoemakeri* can survive periods of drying by burrowing into the clay layer (Gilbert et al. 2018) that typically lines the hypotelminorheic habitat (Staley 2016) a meter or so below the surface.

Finally, we note that the metapopulation model has profound implications for conservation planning. Rather than focus on the above ground aquatic habitat, especially feeder and base-level streams, the focus should be on the forest litter and its health and connectivity. It is not just the seeps occupied at one point in time that need protection, but also unoccupied seeps, that may be occupied later, and the dispersal paths between hypotelminorheic habitats (Fig. 2). The forest litter is not only important for dispersal – it appears that habitat quality is positively correlated with canopy maturity and height (Burch et al. 2022). The metapopulation and its dispersal paths are present in the shallow subsurface—the leaf litter, the hypoelminorheic, and seepage springs.

Conclusions

The biology of the amphipod and isopod species remains largely enigmatic. Their habitat is the most superficial of subterranean habitats, yet many of its inhabitants show strong features of adaptation to aphotic environments, including loss of eyes and pigment. This study strengthens the hypothesis that seeps represent semi-isolated populations that blink on and off through time. Their persistence depends not only on occupied seeps but also on unoccupied seeps and a means of dispersal between them.

Acknowledgements

Collecting in 2022 was done under the auspices of Permit CHOH-2022-0028 from C&O Canal National Historical Park, National Park Service. Thalia Eigen helped with the field work and mapping.

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